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The effects of Octacosanol during taekwondo training: full study protocol and statistical analysis plan

NCT#03557476

Full study protocol

Participants

All procedures reported in this study were conducted and performed according to the protocols approved by Kosin University's Institutional Review Board (KSU IRB 2017-63-HR) and carried out in accordance with the Declaration of Helsinki. This study was also registered with clinicaltrials.gov (NCT03557476). Twenty-six elite male taekwondo athletes (mean age=18±1) with at least 3 years of experience in national taekwondo competitions were recruited from Kosin University. Subject characteristics can be seen in Table 1. All participants were healthy and free from cardiovascular, metabolic, and renal diseases, and all participants received complete information about the study design and provided written informed consent. Participants were asked to abstain from caffeinated drinks, alcohol, and antioxidant supplements during the whole period of the study. All participants had no history of chronic use of antioxidants or nonsteroidal anti-inflammatory drugs (NSAIDs).

Study Design

We used a double-blinded, parallel experimental design. Allocation was stratified for years of training (>3 [n = 13 in OCT group and n=13 in CON group]), and the sequence was generated by a computer-based number. All participants were randomly divided in a 1:1 fashion into two groups: 1) a 5% weight loss with octacosanol intake group (OCT, n=13) and 2) a 5% weight loss with placebo group (CON, n=13) (Figure 1). A power analysis calculation (G*Power 3.1 Universität Kiel, Germany) determined a minimum sample size of 26 would allow the observation of a difference of 3% to 5% between the groups (OCT vs CON) on the primary study outcome variable of body weight with a power of 80%.

Exercise Protocols

Participants performed two hours of a taekwondo training program at the same time of day over six days in a training camp. The intensity of each training sessions was 70-90% of predicted maximum heart rate. Subjects completed a 10-minute dynamic stretching warm-up before training began. Approximately one

hour of the taekwondo training program was devoted to technique training and sparring, while the other hour was spent on physical training. The technique training component of the session included basic techniques, simulated fighting techniques and simulated matches. The physical training component consisted of a circuit training program of interval sprints between various resistance training activities. The participants completed a 10-minute cool-down of static stretching at the end of the training session. The training program can be seen in Table 2. Participants wore body protectors during the entirety of the training session and no injuries were reported. Subjects were allowed to drink water ad libitum during the training sessions.

Dietary Intake

The participants' lifestyle was highly controlled by researchers during the research period, as the study was conducted at a training camp with food intake and physical activity being supervised by trained instructors. Food logs were obtained during the training period. To lose weight, caloric restriction and exercise were used. Weight was measured every day before and after training sessions from each subject. The nutrient profile was 60% carbohydrates, 20% fats, and 20% protein. Before intervention of caloric restriction, nutritionists calculated the subjects' mean caloric intake for the last three days and made a plan for the intake of calories to be progressively reduced by 400 kcal per day, totaling ~2,400 kcal over 6 days. Amount of meals were measured by balance (HANA Instrument CO, LTD, Seoul, South Korea), and provided three times per day using a repeated treatment manner.

Intake of Octacosanol

Two capsules (20-mg each) of 100% refined octacosanol powder from sugar cane (Swanson, Fargo ND, USA) were consumed daily by the octacosanol group for six days, one capsule 30 minutes after morning and afternoon meals. The intake of octacosanol was followed in a double-blind manner with either a 20-mg capsule or a placebo twice a day. The daily concentration of octacosanol was determined from a previous study (Arruzazabala et al. 2002).

Collection of Blood

Before and after the six days of this study, blood samples were collected from each subject. Caffeine and

any drugs related to vascular function were prohibited 24 hours prior to collection of blood samples. 15 ml of blood were collected between 9 and 10 AM by syringe (Bom Medrea Co, LTD, Phnom Penh, Cambodia) after subjects had fasted for 12 hours. The blood samples were immediately centrifuged at 3,000 rpm for 10 min at 4°C, and then plasma was collected and stored at -70°C until analysis.

Blood Lipid Profiles

To examine the levels of plasma lipids, HDL, LDL, TC, and TG were analyzed in duplicate using a chemistry analyzer (HITACHI 7600-210 & HITACHI 7180, Japan). Plasma samples were mixed with butylated hydroxytoluene and R1 reagent and reacted for 60 min at 45°C. After the reaction, the mixed samples were centrifuged at 2,000 rpm for 10 min, and then supernatants were taken. The supernatants were read at 586 nm by a plate reader.

Redox Balance Measurements

Plasma levels of superoxidase dismutase (SOD) were acquired by SOD Assay Kit-WST (Cayman Chemical, Ann Arbor, MI, USA). Samples were mixed with WST working solution, and enzyme working solution, and then the samples were incubated for 20 min at 37°C. The absorbances of the incubated samples were acquired at 450 nm by plate reader. The SOD activity was determined by using a Cobas Mira chemistry analyzer (Roche, Basel, Switzerland). Malondialdehyde (MDA) was measured from BIOXTECHLPO-586 kit (OXIS Health Products, Inc, OR, USA). The plasma samples were incubated with reagent diluent for 5 min at room temperature. The incubated samples were stimulated by Ran-Cell total antioxidant control (Randox, Crumlin, County Antrim, UK), and then analyzed at 340 nm by plate reader. Plasma levels of glutathione peroxidase (GPx) were acquired by GPx Assay Kit (Cayman Chemical, Ann Arbor, MI, USA). Samples were incubated for 20 min at 37°C. The absorbances of the incubated samples were acquired at 450 nm by plate reader. The GPx activity was determined by using a Cobas Mira chemistry analyzer (Roche, Basel, Switzerland). The plasma samples were incubated with reagent diluent for 5 min at room temperature. The incubated samples were stimulated by Ran-Cell total antioxidant control (Randox, Crumlin, County Antrim, UK), and then analyzed at 340 nm by plate reader.

Anthropometry and Body Composition

Body composition was assessed between 9 and 10 AM after a 12-hour fast before and after the six-day training program using an eight-polar tactile-electrode impedance meter (InBody 720; Biospace, Seoul, Korea), which simultaneously recorded bodyweight, fat mass, and fat-free mass. Height was measured with a stadiometer to the nearest 1 cm. Body mass index (BMI) was calculated as weight/height²(Wong et al. 2018).

Statistical analysis plan

Data were analyzed using a 2 x 2 analysis of variance (ANOVA) with repeated measures (group [OCT and CON] x time [before and after 6 days]). If a significant main effect or interaction was noted, a paired *t* test was used for within-group comparisons. Statistical analysis was performed using Statistical Package for the Social Science (SPSS) version 21.0 (BM SPSS Analytics, Armonk, NY). The values were presented as mean and standard error. A value of $P < 0.05$ was considered as statistically significant.